T S14/9/87,88,97,98,142,144,145,148-151,157

14/9/87 (Item 87 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11895241 BIOSIS NO.: 199396059657
Expediting rare variant hemoglobin characterization by combined HPLC/electrospray mass spectrometry
AUTHOR: Witkowska H E; Bitsch F; Shackleton C H L
AUTHOR ADDRESS: Mass Spectrometry Facility, Children's Hosp. Oakland Res. Inst., Oakland, CA 94609, USA**USA
JOURNAL: Hemoglobin 17 (3): p227-242 1993
ISSN: 0363-0269
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Microscale analysis of a variant hemoglobin (Hb) has been achieved by combination of high performance liquid chromatography (HPLC) and electrospray mass spectrometry (ESMS) and the method should be almost universally applicable. We have eliminated preparative scale HPLC of globin chains and semi-preparative HPLC of proteolytic digests which had been used prior to mass spectrometry. Use of microbore HPLC columns reduced the time required for analysis substantially and solvent usage by 100x. Molecular masses of intact globins and masses and sequence information of tryptic peptides could be obtained without collecting and separately analyzing chromatographic fractions. As an example of the use of these methods, we report the characterization of an unknown hemoglobinopathy case that was finally authenticated as Hb P-Galveston (beta-117(G19)His fwdarw Arg), using the following sequence of analyses: 1) ESMS of complete hemolysate, 2) analytical HPLC of globin chains, 3) combined microbore HPLC/ESMS of globin chains to determine their molecular masses, 4) cysteine derivatization and tryptic digestion of mixture of all globins, followed by microbore separation of the peptides, molecular mass determination, and generation of fragmentation patterns allowing confirmation of amino acid sequences. This four-part strategy should allow characterization of almost all variant Hbs. Exceptions would be mutations in regions of globin chains which give rise to small (lt four residues) tryptic peptides, either normal or produced by addition of new tryptic sites and mutations that introduce only minute difference in molecular weight (MW) of tryptic peptides. Since only 10% of each separated peptides is mass analyzed, 90% is available for collection and further structural identification (e.g. by tandem MS or Edman sequencing) if the identity is still in doubt.

DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Blood and Lymphatics--Transport and Circulation; Hematology--Human Medicine, Medical Sciences; Methods and Techniques
BIOSYSTEMATIC NAMES: Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia
ORGANISMS: human (Hominidae)
COMMON TAXONOMIC TERMS: Animals; Chordates; Humans; Mammals; Primates; Vertebrates
MISCELLANEOUS TERMS: LAPAROTOMY; SPLENECTOMY; TORSION OF ECTOPIC SPLEEN
CONCEPT CODES:
10054 Biochemistry methods - Proteins, peptides and amino acids
10064 Biochemistry studies - Proteins, peptides and amino acids
10065 Biochemistry studies - Porphyrins and bile pigments

10504 Biophysics - Methods and techniques
15004 Blood - Blood cell studies
15006 Blood - Blood, lymphatic and reticuloendothelial pathologies
BIOSYSTEMATIC CODES:
86215 Hominidae

14/9/88 (Item 88 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

11859979 BIOSIS NO.: 199396024395
Expression of glycosylated and nonglycosylated human transferrin in mammalian cells: Characterization of the recombinant proteins with comparison to three commercially available transferrins
AUTHOR: Mason Anne B (Reprint); Miller Michael K; Funk Walter D; Banfield David K; Savage Kerry J; Oliver Ronald W A; Green Brian N; Macgillivray Ross T A; Woodworth Robert C
AUTHOR ADDRESS: Dep. Biochem., Univ. Vt. Coll. Med., Burlington, VT 05405, USA**USA
JOURNAL: Biochemistry 32 (20): p5472-5479 1993
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The coding sequence for human serum transferrin was assembled from restriction fragments derived from a full-length cDNA clone isolated from a human liver cDNA library. The assembled clone was inserted into the expression vector pNUT and stably transfected into transformed baby hamster kidney (BHK) cells, leading to secretion of up to 125 mg/L recombinant protein into the tissue culture medium. As judged by mobility on NaDodSO-4-PAGE, immunoreactivity, spectral properties (indicative of correct folding and iron binding), and the ability to bind to receptors on a human cell line, initial studies showed that the recombinant transferrin, is identical to three commercial human serum transferrin samples. Electrospray mass spectrometry (ESMS), anion-exchange chromatography, and urea gel analysis showed that the recombinant protein has an extremely complex carbohydrate pattern with 16 separate masses ranging from 78,833 to 80,802 daltons. Mutation of the two asparagine carbohydrate linkage sites to aspartic acid residues led to the expression and secretion of up to 25 mg/L nonglycosylated transferrin. ESMS, anion-exchange chromatography, and urea gel analysis showed a single molecular species that was consistent with the expected theoretical mass of 75,143 daltons. In equilibrium binding experiments, the nonglycosylated mutant bound to HeLa S-3 cells with the same avidity and to the same extent as the glycosylated protein and the three commercial samples. These studies demonstrate conclusively that carbohydrate has no role in this function.

REGISTRY NUMBERS: 57-13-6: UREA
DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics; Cell Biology; Genetics
BIOSYSTEMATIC NAMES: Cricetidae--Rodentia, Mammalia, Vertebrata, Chordata, Animalia; Hominidae--Primates, Mammalia, Vertebrata, Chordata, Animalia
ORGANISMS: Cricetidae (Cricetidae); human (Hominidae)
COMMON TAXONOMIC TERMS: Nonhuman Vertebrates; Nonhuman Mammals; Rodents; Animals; Chordates; Humans; Mammals; Primates; Vertebrates

CHEMICALS & BIOCHEMICALS: UREA
MISCELLANEOUS TERMS: SIGNAL TRANSDUCTION; SITE-DIRECTED MUTAGENESIS; SOYBEAN TRYPSIN INHIBITOR; TURKEY OVOMUCOID INHIBITOR
CONCEPT CODES:
02506 Cytology - Animal
03506 Genetics - Animal
03508 Genetics - Human
10010 Comparative biochemistry
10064 Biochemistry studies - Proteins, peptides and amino acids
10068 Biochemistry studies - Carbohydrates
10506 Biophysics - Molecular properties and macromolecules
BIOSYSTEMATIC CODES:
86310 Cricetidae
86215 Hominidae

14/9/97 (Item 5 from file: 73)
DIALOG(R)File 73:EMBASE

05523959 EMBASE No: 1993292058
Electrospray mass spectrometric analysis of the domains of a large enzyme: Observation of the occupied cobalamin-binding domain and redefinition of the carboxyl terminus of methionine synthase
Drummond J.T.; Loo R.R.O.; Matthews R.G.
Biophysics Research Division, Department of Biological Chemistry, University of Michigan,Ann Arbor, MI 48109 United States
Biochemistry (BIOCHEMISTRY) (United States) 1993, 32/36 (9282-9289)
CODEN: BICHA ISSN: 0006-2960
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

Cobalamin-dependent methionine synthase from Escherichia coli catalyzes the methylation of homocysteine to form methionine, using methyltetrahydrofolate as the primary methyl donor. We have used electrospray mass spectrometry as a powerful tool for characterizing separable fragments obtained by proteolysis of this monomeric 136.1-kDa enzyme. A central 28.0- kDa domain, reported to bind the cobalamin, has been purified to homogeneity in 30% yield. We were able to detect the domain with bound cobalamin by electrospray mass spectrometry at neutral pH. Mass analysis of a 37.2-kDa carboxyl-terminal domain was grossly inconsistent with either of the two amino acid sequences from previously published DNA sequences. We then used electrospray mass spectrometry to analyze peptides generated by a lysyl endoproteolytic digest of a C-terminal fragment, and we have constructed a peptide map that accounts for >95% of the peptide mass derived from this domain. The correct translational end of this protein (27 residues downstream from the previously predicted ultimate residue) has been established, and sequence conflicts within the two published DNA sequences have been resolved (GenBank Accession Number J04975). Resequencing the DNA near the carboxyl terminus ruled out a frameshifted reading of the DNA and suggested that a cytosine had twice been incorrectly inserted late in the reading frame. The strategies reported here for sequence confirmation, localization of coenzyme- binding regions, and identification of chemically modified peptides within a large protein are potentially applicable to the characterization of many other proteins.

DRUG DESCRIPTORS:
*methionine synthase--endogenous compound--ec
MEDICAL DESCRIPTORS:

*enzyme analysis; *mass spectrometry
article; carboxy terminal sequence; controlled study; dna sequence; frameshift mutation; methylation; nonhuman; priority journal; protein domain
CAS REGISTRY NO.: 37290-90-7 (methionine synthase)
SECTION HEADINGS:
022 Human Genetics
029 Clinical and Experimental Biochemistry

14/9/98 (Item 6 from file: 73)
DIALOG(R)File 73:EMBASE

04554111 EMBASE No: 1991048154
Electrospray mass spectrometry in the clinical diagnosis of variant hemoglobins
Shackleton C.H.L.; Falick A.M.; Green B.N.; Witkowska H.E.
Clinical Mass Spectrometry, Facility, Children's Hosp. Res. Inst., 747 52nd Street,Oakland, CA 94609 United States
Journal of Chromatography - Biomedical Applications (J. CHROMATOGR. BIOMED. APPL.) (Netherlands) 1991, 562/1-2 (175-190)
CODEN: JCBAD ISSN: 0378-4347
DOCUMENT TYPE: Journal; Conference Paper
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

A combination of mass spectrometric (MS) techniques (electrospray MS, liquid secondary ion MS (LSIMS) and MS-MS) has been used for variant hemoglobin (Hb) detection and characterization. Electrospray MS allowed analysis of mixtures of intact globins giving simultaneously the molecular weights (accuracy 1-2 Da) and information about relative amounts of globins present. Currently, 14 Da is the minimum molecular weight difference required experimentally to accurately measure different species present in a mixture of 15-16 kDa proteins. Thus 80 and 79% of the known variants of alpha and beta chains, respectively, can be detected in mixtures with their normal counterparts, including Hb S (molecular weight difference = 30 kDa). Abnormal hemoglobins detected were fractionated by Cinf 4 reversed-phase high-performance liquid chromatography (HPLC), and the separated globin chains (or the mixture of whole precipitated globin) were digested by trypsin. The tryptic peptides were separated by Cinf linf 8 reversed-phase HPLC and analyzed by LSIMS to narrow down the mutation site to a single peptide. The mass measured in LSIMS frequently corresponded to a unique structure, thus giving the unequivocal identification of the mutation and its site. Where there was ambiguity, tandem MS on a Kratos Concept four-sector instrument was used for sequencing the abnormal peptide. The practical use of the methodologies presented is illustrated through analysis and identification of Hb G-San Jose, Hb Stanleyville II, Hb S and Hb Willamette.

DRUG DESCRIPTORS:
*hemoglobin--endogenous compound--ec
MEDICAL DESCRIPTORS:
*chemical analysis; *mass spectrometry
conference paper; human; molecular weight; priority journal
CAS REGISTRY NO.: 9008-02-0 (hemoglobin)
SECTION HEADINGS:
025 Hematology
029 Clinical and Experimental Biochemistry

14/9/142 (Item 44 from file: 154)
DIALOG(R)File 154:MEDLINE(R)

09886362 PMID: 8280919
Direct peptide mapping of sickle-cell hemoglobin using electrospray mass spectrometry.
Sakairi M
Central Research Laboratory, Hitachi Ltd., Tokyo, Japan.
Rapid communications in mass spectrometry - RCM (ENGLAND) Dec 1993, 7 (12) p1108-12, ISSN 0951-4198--Print Journal Code: 8802365
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS
Direct peptide mapping of sickle-cell hemoglobin (Hb S) using electrospray mass spectrometry is demonstrated to be a rapid, sensitive and useful technique for providing information concerning the location of variation. By fully comparing electrospray mass spectra of digests of sickle-cell and normal hemoglobins, using L-1-tosylamide-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin, the point mutation of B1 fragment of beta-chain in Hb S can be confirmed, taking into consideration that all the other fragments are completely identical in the two proteins.
Descriptors: *Hemoglobin, Sickle--genetics--GE; *Peptide Mapping--methods --MT; Amino Acid Sequence; Molecular Sequence Data; Spectrum Analysis, Mass --methods--MT
CAS Registry No.: 0 (Hemoglobin, Sickle)
Record Date Created: 19940217
Record Date Completed: 19940217

14/9/144 (Item 46 from file: 154)
DIALOG(R)File 154:MEDLINE(R)

09757329 PMID: 7764097
Electrospray mass spectrometry characterization of post-translational modifications of barley alpha-amylase 1 produced in yeast.
Sogaard M; Andersen J S; Roepstorff P; Svensson B
Department of Chemistry, Carlsberg Laboratory, Copenhagen Valby, Denmark.
Bio/technology (Nature Publishing Company) (UNITED STATES) Oct 1993, 11 (10) p1162-5, ISSN 0733-222X--Print Journal Code: 8309273
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: BIOTECHNOLOGY
We have used electrospray mass spectrometry (ESMS) in combination with protein chemistry and genetics to delineate post-translational modifications in yeast of barley alpha-amylase 1 (AMY1), a 45 kD enzyme crucial for production of malt, an important starting material in the manufacture of beer and whisky. In addition to signal peptide processing these modifications are: (1) removal of C-terminal Arg-Ser by Kex1p, (2) glutathionylation of Cys95, (3) O-glycosylation, and (4) additional degradation of the C-terminus.
Descriptors: *Hordeum--enzymology--EN; *Protein Processing, Post-Translational; *Saccharomyces cerevisiae--genetics--GE; *Spectrum

Analysis, Mass--methods--MT; *alpha-Amylase--metabolism--ME; Base Sequence; Blotting, Western; Glutathione--metabolism--ME; Molecular Sequence Data; Mutation; Plasmids; Protein Folding; Recombinant Proteins--chemistry--CH; Recombinant Proteins--metabolism--ME; Research Support, Non-U.S. Gov't; alpha-Amylase--chemistry--CH; alpha-Amylase--genetics--GE
CAS Registry No.: 0 (Plasmids); 0 (Recombinant Proteins); 70-18-8 (Glutathione)
Enzyme No.: EC 3.2.1.1 (alpha-Amylase)
Record Date Created: 19931123
Record Date Completed: 19931123

14/9/145 (Item 47 from file: 154)
DIALOG(R)File 154:MEDLINE(R)

09756081 PMID: 8379946
Demonstration by electrospray mass spectrometry that the peptidyldipeptidase activity of cathepsin B is capable of rat cathepsin B C-terminal processing.
Rowan A D; Feng R; Konishi Y; Mort J S
Joint Diseases Laboratory, Shriners Hospital for Crippled Children, Montreal, Quebec, Canada.
Biochemical journal (ENGLAND) Sep 15 1993, 294 (Pt 3) p923-7, ISSN 0264-6021--Print Journal Code: 2984726R
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS; Toxbib
Electrospray mass spectrometric techniques were used to demonstrate that mature (single-chain) recombinant rat cathepsin B is capable of sequentially removing the three dipeptides which comprise the C-terminal extension of the proenzyme. A pepsin-cleaved form of a non-active mutant recombinant rat procathepsin B (Cys-29-Ser) was used as a 'substrate' to study C-terminal processing by mature cathepsin B. The results indicate that the first two residues (Arg-Phe) are removed efficiently, while the remaining four (Gln-Tyr-Trp-Gly), particularly the final two, are much more resistant to proteolysis. These cleavages were pronounced at pH 5.0 compared with pH 6.0, in agreement with the lower pH optimum for cathepsin B exopeptidase activity reported previously. From this example of the peptidyldipeptidase activity of cathepsin B we conclude that removal of the C-terminal extension may occur in any intracellular compartment where active cathepsin B is found.
Descriptors: *Cathepsin B--metabolism--ME; *Endopeptidases--metabolism --ME; Amino Acid Sequence; Animals; Base Sequence; Enzyme Precursors --metabolism--ME; Molecular Sequence Data; Mutagenesis, Site-Directed; Oligodeoxyribonucleotides--chemistry--CH; Pepsin A--metabolism--ME; Protein Processing, Post-Translational; Rats; Research Support, Non-U.S. Gov't; Spectrum Analysis, Mass; Substrate Specificity
CAS Registry No.: 0 (Enzyme Precursors); 0 (Oligodeoxyribonucleotides)
Enzyme No.: EC 3.4.- (Endopeptidases); EC 3.4.22.1 (Cathepsin B); EC 3.4.23.1 (Pepsin A)
Record Date Created: 19931019
Record Date Completed: 19931019

14/9/148 (Item 50 from file: 154)
DIALOG(R)File 154:MEDLINE(R)

09643630 PMID: 8497198

Properties of FNR proteins substituted at each of the five cysteine residues.

Green J; Sharrocks A D; Green B; Geisow M; Guest J R
Krebs Institute, Department of Molecular Biology and Biotechnology, University of Sheffield, UK.

Molecular microbiology (ENGLAND) Apr 1993, 8 (1) p61-8, ISSN 0950-382X--Print Journal Code: 8712028
Publishing Model Print
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: MEDLINE; Completed
Subfile: INDEX MEDICUS; Toxbib

FNR is a transcriptional regulator controlling the expression of a number of Escherichia coli genes in response to anoxia. It is structurally-related to CRP (the cyclic AMP receptor protein) except for the presence of a cysteine-rich N-terminal extension, which may form part of an iron-binding, redox-sensing domain in FNR. Site-directed substitution has previously shown that four of the cysteine residues (C20, C23, C29 and C122) are essential for FNR function, whereas the fifth (C16) is not. The FNR protein exists in two forms separable by non-reducing SDS-PAGE, and in studies with altered FNR proteins containing single substitutions at each of the five cysteine residues it was concluded that the faster-migrating form (FNR(27)), possesses an intramolecular disulphide bond linking C122 to one of the cysteines near the N-terminus. FNR(27) was more abundant in aerobic cells but the physiological significance of this was not established. Footprint studies indicated that FNR proteins lacking essential cysteine residues are impaired in their ability to protect FNR sites in the ndh promoter. The non-essential cysteine residue (C16) was identified as the source of the most reactive sulphydryl group and all of the inactive proteins exhibited different sulphydryl reactivities. The iron content of the C122A-substituted protein was much reduced but those of the other proteins were less affected. Electrospray mass spectrometry confirmed the accuracy of the gene-derived amino acid composition of FNR with a mutant protein and it showed that a fraction of the wild-type protein may carry a 78 Da substituent which could not be removed with dithiothreitol or beta-mercaptoethanol.

Descriptors: *Bacterial Proteins--genetics--GE; *Cysteine; *Escherichia coli--genetics--GE; *Escherichia coli Proteins; *Iron-Sulfur Proteins; *Transcription Factors--genetics--GE; Aerobiosis; Bacterial Proteins --metabolism--ME; Comparative Study; Iron--metabolism--ME; Mutagenesis, Site-Directed; Oxidation-Reduction; Research Support, Non-U.S. Gov't; Spectrum Analysis, Mass; Sulfhydryl Compounds--metabolism--ME; Transcription Factors--metabolism--ME; Transcription, Genetic
CAS Registry No.: 0 (Bacterial Proteins); 0 (Escherichia coli Proteins); 0 (FNR protein, E coli); 0 (Iron-Sulfur Proteins); 0 (Sulfhydryl Compounds); 0 (Transcription Factors); 52-90-4 (Cysteine); 7439-89-6 (Iron)
Record Date Created: 19930622
Record Date Completed: 19930622

14/9/149 (Item 51 from file: 154)

09617247 PMID: 8476748

Structure-function studies of human aromatase.

Chen S; Zhou D; Swiderek K M; Kadohama N; Osawa Y; Hall P F

Division of Immunology, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

Journal of steroid biochemistry and molecular biology (ENGLAND) Mar 1993, 44 (4-6) p347-56, ISSN 0960-0760--Print Journal Code: 9015483

Contract/Grant No.: CA 33572; CA; NCI; CA 44735; CA; NCI; HD 04949; HD; NICHD

Publishing Model Print

Document type: Journal Article; Review

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS; Toxbib

Site-directed mutagenesis experiments have been carried out to determine the structure-function relationship of human aromatase. By sequence comparison, the region in aromatase that corresponds to the distal helix of cytochrome P-450cam has been identified to be Gln-298 to Val-313. Eight aromatase mutants with changes in this region, i.e. C299A, E302L, P308F, D309N, D309A, T310S, T310C, and S312C, have been generated using a mammalian cell stable-expression system. The results from site-directed mutagenesis studies indicate that the region containing Gln-298 to Val-313 is indeed a very important part of the active site of aromatase. The catalytic properties of P308F, D309N, and D309A have been examined in detail and are discussed. Active site-directed labeling is also an important approach to investigate the structure-function relationship of aromatase. HPLC-linked electrospray mass spectrometry is indicated as a useful technique for the characterization of active site-directed probe-modified enzyme. The mass spectral analysis of aromatase suggests that aromatase is glycosylated. (34 Refs.)

Descriptors: *Aromatase--genetics--GE; *Aromatase--metabolism--ME; Amino Acid Sequence; Animals; Aromatase--chemistry--CH; Binding Sites; Cell Line; Humans; Models, Molecular; Molecular Sequence Data; Mutagenesis, Site-Directed; Protein Structure, Secondary; Research Support, U.S. Gov't, P.H.S.; Sequence Homology, Amino Acid; Transfection

Enzyme No.: EC 1.14.14.1 (Aromatase)

Record Date Created: 19930526

Record Date Completed: 19930526

14/9/150 (Item 52 from file: 154)

DIALOG(R)File 154:MEDLINE(R)

09144339 PMID: 1733771

A study of D52S hen lysozyme-GlcNAc oligosaccharide complexes by NMR spectroscopy and electrospray mass spectrometry.

Lumb K J; Aplin R T; Radford S E; Archer D B; Jeenes D J; Lambert N; MacKenzie D A; Dobson C M; Lowe G

Inorganic Chemistry Laboratory, University of Oxford, UK.

FEBS letters (NETHERLANDS) Jan 20 1992, 296 (2) p153-7, ISSN 0014-5793--Print Journal Code: 0155157

Publishing Model Print; Erratum in FEBS Lett 1993 Mar 22;319(3) 292

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

The production of a mutant hen lysozyme is described in which Asp-52, one of the catalytically important residues, is replaced by Ser. The mutant

enzyme has very low catalytic activity but NMR studies show that its structure is closely similar to that of the wild-type protein. NMR experiments also show that well defined complexes are formed with GlcNAc4 and GlcNAc6 bound in the active site of the mutant enzyme. These complexes have been examined using electrospray mass spectrometry (ESMS). The most intense peaks arise from the uncomplexed protein indicating that dissociation takes place in the mass spectrometer under the conditions used here. Peaks from minor species corresponding to complexes between the protein and the oligosaccharides are, however, also observed. The possibility that the latter arise from novel covalent enzyme-saccharide complexes is discussed.

Descriptors: *Muramidase--chemistry--CH; *Mutation; Acetylglucosamine --chemistry--CH; Acetylglucosamine--metabolism--ME; Animals; Binding Sites ; Chickens; Magnetic Resonance Spectroscopy; Muramidase--genetics--GE; Muramidase--metabolism--ME; Oligosaccharides--chemistry--CH; Oligosacchar ides--metabolism--ME; Research Support, Non-U.S. Gov't; Spectrum Analysis, Mass

CAS Registry No.: 0 (Oligosaccharides); 7512-17-6 (Acetylglucosamine)

Enzyme No.: EC 3.2.1.17 (Muramidase)

Record Date Created: 19920304

Record Date Completed: 19920304

14/9/151 (Item 53 from file: 154)

DIALOG(R)File 154:MEDLINE(R)

08763416 PMID: 2134187

Tandem mass spectrometry in the clinical analysis of variant hemoglobins.

Falick A M; Shackleton C H; Green B N; Witkowska H E

Department of Pharmaceutical Chemistry, University of California, San Francisco.

Rapid communications in mass spectrometry - RCM (ENGLAND) Oct 1990, 4 (10) p396-400, ISSN 0951-4198--Print Journal Code: 8802365

Contract/Grant No.: HL20985; HL; NHLBI; RR 01614; RR; NCRR

Publishing Model Print

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: MEDLINE; Completed

Subfile: INDEX MEDICUS

A combination of mass spectrometric techniques (electrospray mass spectrometry, liquid secondary-ion mass spectrometry (LSIMS), tandem mass spectrometry) has been used for variant hemoglobin detection and characterization. Electrospray mass spectrometry allowed analysis of mixtures of intact globins giving the molecular weights (accuracy 1-2 Da), and information about relative amounts of globins present, simultaneously. Abnormal hemoglobins detected in this way and by other means (screening, clinical symptoms) were fractionated by C-4 reverse phase high-performance liquid chromatography (HPLC), and the separated globin chains (or the mixture of whole precipitated globin) were digested with trypsin. The tryptic peptides were separated by C-18 reverse phase HPLC and analysed by LSIMS to narrow down the mutation site to a single peptide. In some instances, the molecular weight of a variant peptide was sufficient to determine the mutation uniquely. When molecular weight information alone was insufficient to identify the mutation and its site, the peptide was sequenced by tandem mass spectrometry on a 4-sector instrument. In cases where more than one possible mutation site was present in the peptide and the mutation resulted in a change of only 1 Da in the peptide mass, the resolution and mass measurement accuracy of the 4-sector machine were

essential in determining the correct sequence. The practical application of the methodologies presented is illustrated by the identification and analysis of Hb G-San Jose, Hb Willamette and D-Iran.
Descriptors: *Hemoglobins, Abnormal--analysis--AN; Humans; Infant; Research Support, Non-U.S. Gov't; Research Support, U.S. Gov't, Non-P.H.S.; Research Support, U.S. Gov't, P.H.S.; Spectrum Analysis, Mass --instrumentation--IS; Spectrum Analysis, Mass--methods--MT
CAS Registry No.: 0 (Hemoglobins, Abnormal); 39354-88-6 (hemoglobin D Iran); 61840-07-1 (hemoglobin Willamette); 9034-75-7 (hemoglobin G San Jose)
Record Date Created: 19920421
Record Date Completed: 19920421

14/9/157 (Item 5 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0157003 DBR Accession No.: 93-15055
Mass spectrometric analysis of biotechnology products - fast atom bombardment mass spectroscopy and electrospray mass spectroscopy
AUTHOR: Greer F M; Savoy L A
CORPORATE AFFILIATE: M-Scan
CORPORATE SOURCE: M-Scan Ltd., Silwood Park, Sunninghill, Ascot, Berkshire, SL5 7PZ, UK.
JOURNAL: Genet.Eng.Biotechnol. (13, 2, 105-10) 1993
CODEN: 4357H
LANGUAGE: English
ABSTRACT: The characterization of the structure of genetically engineered bio-pharmaceutical products presents a challenge for analytical chemists. Modern advances in MS have led to the development of several techniques such as FAB-MS and electrospray-MS (ES-MS) as powerful tools for solving complex protein and glycoprotein structural problems. These procedures are complementary to classical Edman chemistry but in addition have the ability to observe and assign aspects of structure such as blocking groups at the N-terminus or micro-heterogeneity at the C-terminus which are difficult to address using conventional techniques. In the analysis of recombinant products, ES and FAB mapping can be used to provide sequence conformation, and detect errors of translation, mutation, insertion or deletion. Importantly, FAB-MS techniques can be used not only to locate sites of glycosylation, but also to determine the basic carbohydrate structure(s) present at each site. These techniques can be utilized at research, quality control and regulatory approval stages. (8 ref)
DESCRIPTORS: FAB-mass spectroscopy, ES-MS analysis of recombinant product (Vol.12, No.26)
SECTION: ANALYSIS-Sensors and Analysis; GENETIC ENGINEERING AND FERMENTATION-Nucleic Acid Technology (C1,A1)
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